# A Common Keratin 5 Gene Mutation in Epidermolysis Bullosa Simplex—Weber-Cockayne

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**The Weber-Cockayne subtype of epidermolysis bullosa simplex is an inherited skin-fragility disorder characterized by basal keratinocyte lysis and epidermal blistering confined primarily to the hands and feet. The disorder results from a mutation in either the keratin 5 or keratin 14 gene, which encode the peptide components of the obligate heterodimeric keratin intermediate filaments of the basal cell. We have determined that a T → G substitution mutation in keratin 5, which results in a Ile → Ser change at codon 161, is common among patients with the Weber-Cockayne disease variant, accounting for six of 13 cases tested. The observed high frequency of this mutation may result from either a mutational hot spot or a founder effect. The potential utility of this common mutation in confirming disease status in some at-risk individual is discussed.** ***Key words: intermediate filaments/blistering/epidermal/diagnosis. J Invest Dermatol 104:877–879, 1995***

Epidermolysis bullosa simplex (EBS) is a group of inherited skin disorders in which mild mechanical trauma leads to basal keratinocyte lysis and subsequent blister formation in the epidermis (reviewed in [1]). Three major clinical subtypes of EBS have been defined based on severity of findings and skin histopathology. The Weber-Cockayne subtype (EBS-WC) has the mildest presentation with blistering confined primarily to the hands and feet, whereas the Koebner subtype of the disorder has generalized blistering. The generalized blistering pattern of the third subtype, Dowling-Meara, is distinguished further by the presence of circumscribed clumps of keratin filaments evident in the basal cells by electron microscopy. Generally, the EBS disorders are inherited in an autosomal dominant fashion, although a few families with recessive inheritance have been reported [2,3].

Recently, the molecular basis of the three major EBS subtypes have been found to reside in defects that disrupt the assembly, structure, and/or function of the keratin intermediate filament (KIF) skeleton of the basal keratinocyte. The primary structural components of the basal cell KIF are the coexpressed peptides keratin 5 and keratin 14, which form the coil-coiled obligate heterodimers that are the building blocks of the KIF (reviewed in [4]). Mutational analyses of affected individuals have demonstrated that each of the three major EBS subtypes can result from a single mutation in either KRT5, the gene that encodes the keratin 5 peptide, or KRT14, the gene encoding the keratin 14 peptide [2,3,5–13].

To identify additional EBS mutations for studies of genotype/phenotype correlation, we have begun to screen 13 unrelated EBS patients for both previously reported and novel mutations in the KRT5 and KRT14 genes. Here we describe that a previously identified KRT5 mutation is common among EBS-WC patients and discuss its potential utility in confirming disease status in some at-risk individuals.

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## MATERIALS AND METHODS

**Families** EBS-WC families were ascertained through the National Epidermolysis Bullosa Registry, by advertisement by the Dystrophic Epidermolysis Bullosa Research Association, and by referral directly to one of the authors (VPS). Isolated cases with sporadic disease were categorized as having the WC variant of EBS if their blisters were limited to hands and feet and areas of specific excess trauma (e.g., under tight belts, on inner thighs after prolonged horseback riding) beyond infancy. In pedigrees in which most members had lesions limited to palms and soles, an occasional individual had more widespread blistering. These persons were also labeled as EBS-WC. Similarly, individuals in whom blistering was limited primarily to palms and soles, but in whose family most affected individuals had wide spread blistering, were labeled as EBS-Koebner. These determinations were arbitrary and based on best clinical judgement. Thirteen EBS-WC families of northern European ancestry, comprised of 121 individuals including 65 affected, were studied. Peripheral blood leukocytes from affected and unaffected family members were immortalized by transformation with Epstein-Barr virus [14]. Analyses of three highly polymorphic loci in each family were consistent with paternity as stated (data not shown).

**Mutation Identification** KRT5 exon 1, which includes basepair 482 (numbering assumes position 1 is the first base of the initiating AUG), was amplified by the polymerase chain reaction with primers 5′-GCTATGGCTTTGGAGGTGGT-3′ (bp 672–691 according to the numbering in [15]) and 5′-CCTTCTTTCTCTCTCTTTGGC-3′ (bases 49–69 of intron 1 [P. Ehrlich and K. Stephens, unpublished]). A 50-$\mu$l reaction contained 250 ng genomic DNA, 0.5 $\mu$M each primer, 0.8 mM deoxynucleotide triphosphates, 1 × polymerase chain reaction buffer with 1.5 mM $Mg^{++}$ (Perkin-Elmer), and 2 U AmpliTaq polymerase (Perkin-Elmer). Thirty-five cycles were performed at 94°C for 45 seconds, 55°C for 30 seconds, and 72°C for 45 seconds. 1.5 U of FokI (Boehringer-Mannheim) were added to 30 $\mu$l of the product, digested overnight at 37°C, and electrophoresed through a 3% NuSieve gel (FMC, Rockland, ME).

## RESULTS

Recently, two unrelated EBS-WC patients were reported to have the same T→G substitution mutation at basepair 482 (designated

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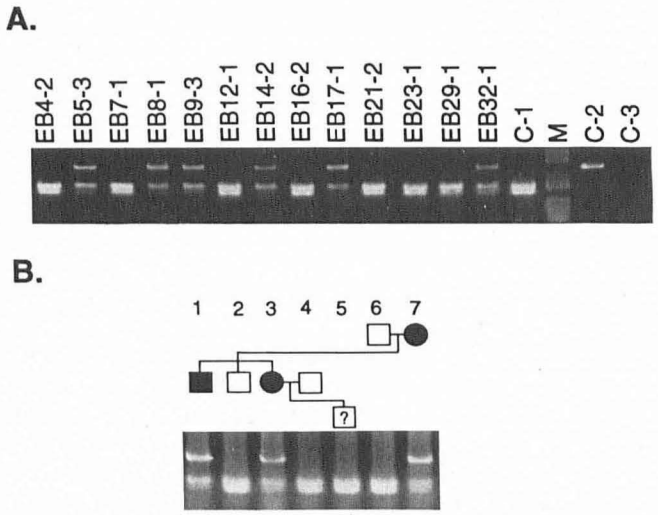


**Figure 1. The T482G mutation occurred in six of 13 EBS-WC families.** The T482G mutation, which obliterates a FokI restriction site [11], was detected by FokI digestion of KRT5 exon 1 amplified DNA as described. *A*) FokI digests of unrelated EBS-WC patients and C-1, an unrelated, unaffected control individual. Lane C-2 is uncut PCR product amplified from individual C-1; lane C-3 is the PCR reaction in the absence of template DNA. The undigested product is 343 bp; digestion produces two products of 185 and 158 bp in length. M, size marker of HaeIII digested pBR322. *B*) Mutational analysis of family EB5. Individual 5, a 16-month–old boy, was asymptomatic. Symbols are as described in **Fig 2**.

T482G) of the KRT5 gene predicting an isoleucine to serine substitution in codon 161 [11]. To determine if this mutation was common among EBS-WC patients, we screened an affected individual from each of 13 unrelated families. Of the 13 patients screened, six carried the T→G substitution as indicated by the obliteration of a FokI restriction site ([11]; **Fig 1*A***). Direct sequencing of patient EB5-3 confirmed the T482G substitution mutation was responsible for the FokI restriction pattern (data not shown).

To determine if the T482G mutation co-segregated with the EBS-WC disease phenotype, 68 family members of the six patients carrying the mutation were tested **(Fig 2)**. In all cases, only affected family members tested positive for obliteration of the FokI site, as illustrated for family EB5 in **Fig 1*B***. In this family, a 16-month–old asymptomatic child (individual 5) of an affected mother was also tested. As shown, the FokI digested DNA from the child was cleaved by FokI, indicating that he does not carry the T482G mutation that is responsible for EBS-WC in his family.

KRT5 exon 1 amplification and FokI digestion of genomic DNA from 25 unrelated, unaffected control individuals revealed no alleles that had lost the restriction site (data not shown). These results, in addition to similar results on 78 individuals reported previously [11], demonstrate that the obliteration of the FokI restriction site is not due to a common polymorphism.

## DISCUSSION

We have determined that the T482G mutation in codon 161 of the KRT5 gene is common among EBS-WC patients, accounting for six of 13 cases tested. At the present time, we cannot determine whether the high frequency of T482G is the result of a mutational hotspot or a founder effect. No instances of new mutation were among the cases studied **(Fig 2)**. Because all six of the families carrying this mutation are of northern European ancestry, and this disease has no significant effect on fitness, it is possible that the *de novo* mutation occurred in a single ancestral individual (founder). The resources required to distinguish between these two possibilities, additional EBS-WC families with the T482G mutation and multiple highly polymorphic sites within 1 centiMorgan of KRT5, are not available at the present time.

In family EB5, DNA studies confirmed the unaffected status of a 16-month–old child. In this particular family, where affected individuals invariably manifested blisters by three or four months of age, clinical criteria alone predicted the child was at low risk to have inherited EBS-WC. However, in EBS-WC families in general, onset of blistering may be delayed until ambulation (10–15 months) or even later in childhood or the teen years, and the uncertainty can be a source of anxiety for the family. In the subset of EBS-WC families with the T482G mutation, it is now possible to determine unambigously the disease status of a newborn or a young child.

Two frequent mutations that predict the production of defective keratin molecules have now been identified as a cause for EBS.

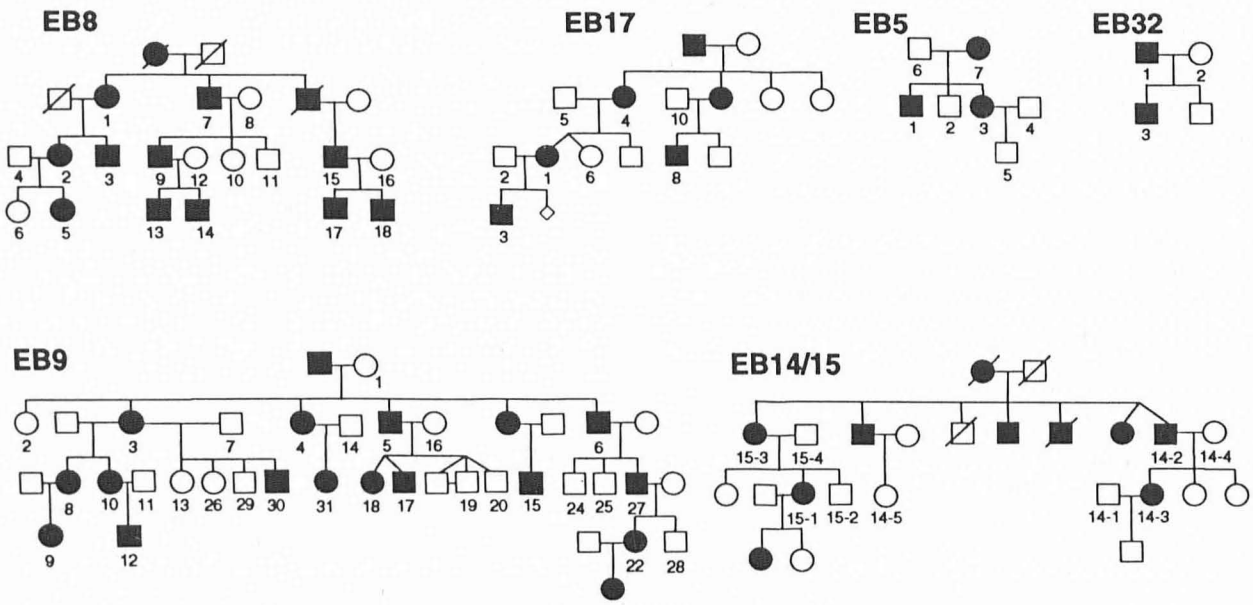


**Figure 2. Six EBS-WC families with the T482G mutation of KRT5.** Individuals tested for the mutation are designated by number. Male (*square*), female (*circle*), sex not given (*diamond*), affected with EBS-WC (*solid symbols*), unaffected (*open symbols*), deceased (*slashed symbols*).

First, a mutational hotspot in KRT14 that substitutes a Cys or His for an Arg in codon 125 that accounted for 50% of EBS–Dowling-Meara cases [7]. Second, the T482G mutation in codon 161 of the KRT5 gene described here that accounted for 45% of EBS-WC cases. Although the hypermutatility of KRT14 codon 125 has been attributed to spontaneous deamination of methylated CpG dinucleotides [5,7], no such sites occur in codon 161 of KRT5. The defective keratin molecules produced by the common mutations most likely act in a dominant-negative fashion to disrupt KIF assembly or function, as do other KRT5 or KRT14 mutations underlying dominant EBS disease [5,16].

Mutational analyses of additional EBS families are necessary to determine the effects of specific mutated residues or domains and/or altered ratios of defective to normal peptides on KIF organization and function. The secondary structure of keratins 5 and 14 consists of a central rod domain flanked by head and tail regions. The central rod is comprised of four α-helical subdomains (1A, 1B, 2A, 2B) that are separated by three linker regions (reviewed in [4]). Initially, the location of keratin mutations in patients and *in vitro* keratin assembly experiments suggested that amino acid substitutions in the beginning of domain 1A and the end of domain 2B were the most disruptive to KIF function, resulting in KIF clumping and severe disease [12,16,17]. For example, the KRT14 codon 125 mutation responsible for EBS–Dowling-Meara maps at the tenth codon of domain 1A and the KRT5 T482G mutation responsible for EBS-WC maps in the head region. However, correlations between genotype and phenotype are more complex that originally suspected. Recently, we have identified a mutation in the beginning of domain 1A that does not result in KIF clumping and severe EBS disease, but rather the EBS-Koebner phenotype even when present in the homozygous state [13]. The identification of additional mutations that alter keratin structure will facilitate investigations in assembly and function of keratin intermediate filaments.

*Note Added in Proof:* Recently, we have identified the T482G mutation in three of five additional EBS-WC families of Northern European ancestry.

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